

FIG. 2

SEQ ID: 1

SEQ ID: 2

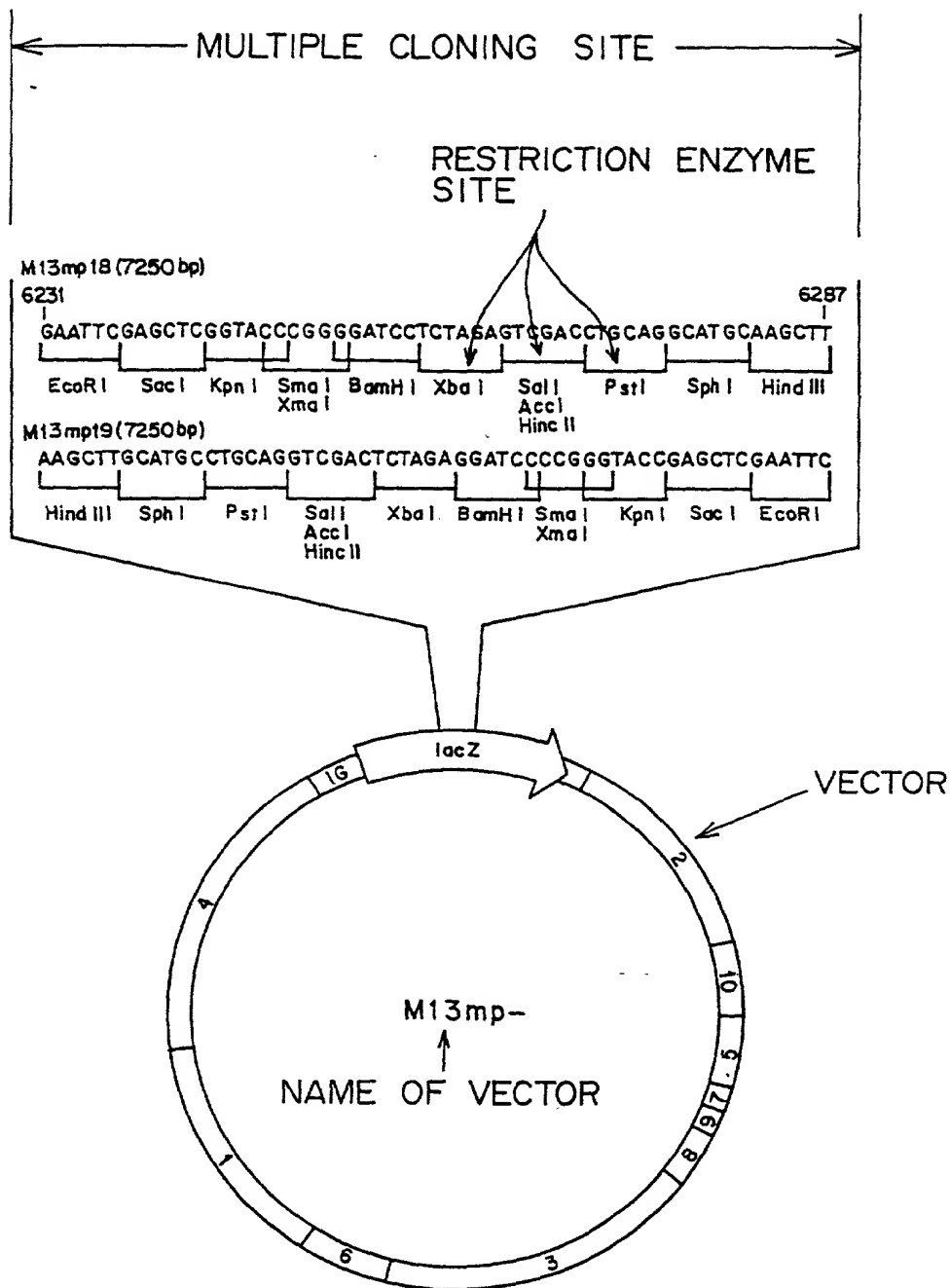
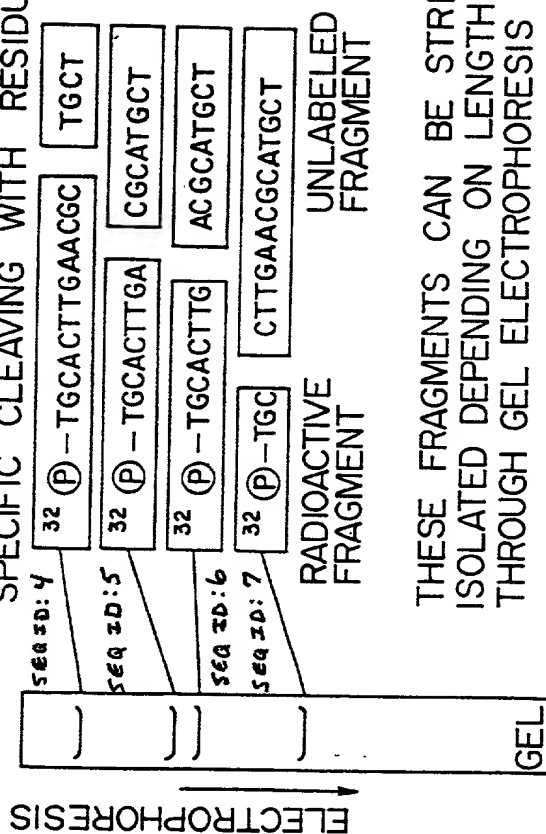


FIG. 3

DNA FRAGMENT LABELED WITH ^{32}P AT 5' EDGE

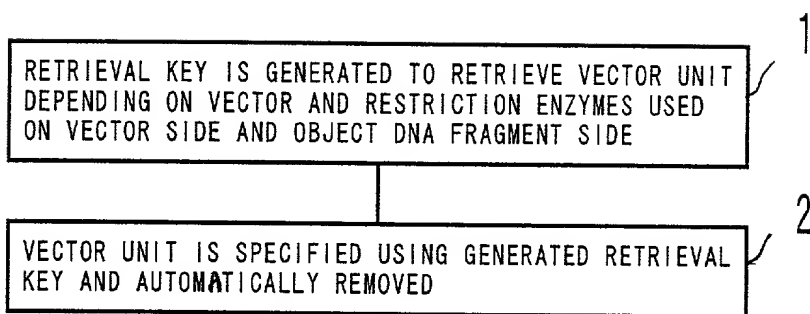
SEQ ID: 3 ^{32}P - TGCACCTTGAACGCATGCT

RADIOACTIVE FRAGMENTS OF VARIOUS LENGTHS THROUGH CHEMICAL PROCESS OF SPECIFIC CLEAVING WITH RESIDUAL BASE A



THESE FRAGMENTS CAN BE STRICTLY ISOLATED DEPENDING ON LENGTH THROUGH GEL ELECTROPHORESIS

FIG. 4



F I G . 5

```

graph TD
    S6[INPUT OF VECTOR  
TYPE OF USED VECTOR IS SELECTED FROM VECTOR LIST] --> S7[INPUT OF RESTRICTION ENZYME  
USED RESTRICTION ENZYME IS SELECTED FROM RESTRICTION ENZYME LIST]
    S7 --> S8[VECTOR UNIT SPECIFICATION PROGRAM  
RETRIEVAL KEY IS GENERATED AND RETRIEVED FROM VECTOR AND RESTRICTION ENZYME INFORMATION  
HOMOLOGY CHECK IS MADE BETWEEN RETRIEVAL KEY AND MULTIPLE CLONING SITE TO SELECT VECTOR UNIT]
    S8 --> S9[REMOVAL OF VECTOR UNIT  
VECTOR UNIT SPECIFIED BY VECTOR UNIT SPECIFICATION PROGRAM IS REMOVED]

```

F I G. 6

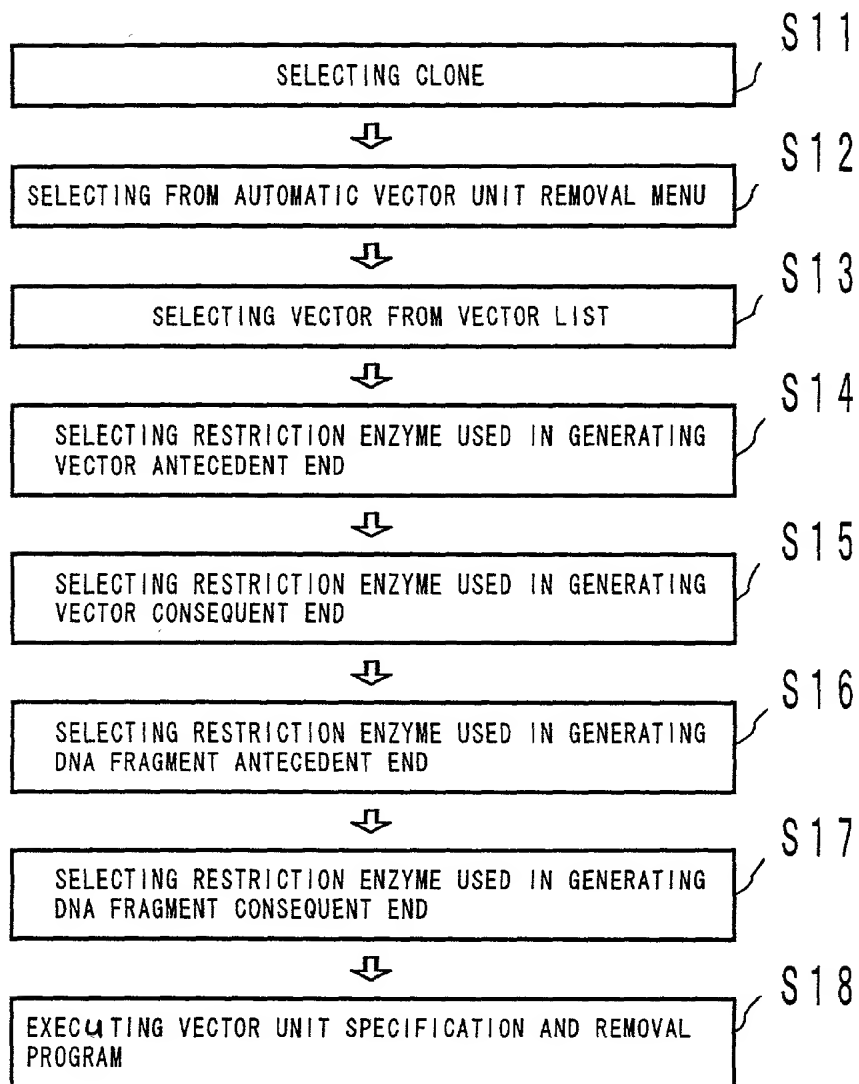


FIG. 7

M13MP18
M13MP19
PBR322
PSL1180
PSL1190
PT7T318U
PT7T319U
PTZ18R
PTZ19R
PUC18
PUC19, ETC.

FIG. 8

VECTOR DB FORMAT

```

>ID
PUC18
>SEQ ID: 8
TCGCGCGTTCGGTGATGACGGTGAAAACTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGAT
GCGGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGCGGGCTGGCTTAACATATGCGGCATCAGA
GCAGATTGTACTGAGAGTGACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGCC
ATTGCGCATTGAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGG
GGATGTGCTGCAAGGCGATTAAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAACGACGCGCCAGTGCCAA
GCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCT
GTGTGAAATTGTTATCCGCTCACAAATCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATG
AGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTCCAGTCGGGAAACCTGTGCTGCCAGCTGCATTAAT
GAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTGGGCGCTCTTCGCTTCTCGCTCACTGACTCGCTGCGCTCG
GTCGTTGCGGCTGCGGGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGTTATCCACAGAATCAGGGGATAACGCAGG
AAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCC
GCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACGAGCG
TTTTCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTC
GGGAAGCGTGGCGCTTTCTAAAGCTCAGCGTGTAGGTATCTCAGTTGCGGTGTAGGTGCTTCCAGCTGGGCTGTG
TGCACGAACCCCGCTTCAGCCGACCGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAAACCCGTAAGACACGAC
TTATCGCCACTGGCAGCAGCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTG
GTGGCCTAACTACGGCTACACTAGAAGAACAGTATTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAG
TTGGTAGCTCTTGATCCGGCAAAACAAACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGA
AAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAACTCACGTTAAGGGAT
TTTGGTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAAGTGTAAATCAATCTAAAGTA
TATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCA
TCCATAGTTGCCTGACTCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGAT
ACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTC
CTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCGGGAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTG
CGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTTGGTATGGCTTCATTCAGCTCCGGTTCCCA
ACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTAGAA
GTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTATGCCATCCGTAAGATGC
TTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGCGCTC
AATACGGGATAAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATATTGAAAAAGCTTCTTCGGGGCGAAAACTCT
CAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTACTTTT
ACCAGCGTTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACAGGAAATGTTGAAT
ACTCATACTCTTCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTGAATGTA
TTAGAAAAATAAACAATAGGGGTTCCGCGCACATTTCCCGAAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATC
ATGACATTAACCTATAAAAAATAGGCGTATCAGGAGCCCTTTTCGT
>MULTI
399..450

```

FIG. 9

VECTOR SIDE

HIND III

SPH I

PST I

SAL I

ACC I

HINC II

XBA I

BAMH I

SMA I

XMA I

KPN I

SAC I

ECOR I

OBJECT DNA
FRAGMENT SIDE

HINDIII

SPH I

PST I

SAL I

ACC I

HINC II

XBA I

BAMH I

SMA I

XMA I

KPN I

SAC I

ECOR I

OTHER RESTRICTION
ENZYME

. . .

FIG. 11

S 2 1

S 2 2

S 23

S 2 4

S 25

FIG. 12

WHEN SINGLE-STRANDED AREA IS FOUND ON 3' SIDE

| | | | | |
|------------------------|--------|---------|--------|----|
| STRAND A 5' | AREA A | AREA B3 | AREA C | 3' |
| STRAND B 3' | AREA C | AREA B3 | AREA A | 5' |
| ← RESTRICTION ENZYME → | | | | |
| SITE | | | | |

FIG. 13A

WHEN NO SINGLE-STRANDED AREA IS FOUND

| | | | |
|------------------------|--------|--------|----|
| STRAND A 5' | AREA A | AREA C | 3' |
| STRAND B 3' | AREA C | AREA A | 5' |
| ← RESTRICTION ENZYME → | | | |
| SITE | | | |

FIG. 13B

WHEN SINGLE-STRANDED AREA IS FOUND ON 5' SIDE

| | | | | |
|------------------------|--------|---------|--------|----|
| STRAND A 5' | AREA A | AREA B5 | AREA C | 3' |
| STRAND B 3' | AREA C | AREA B5 | AREA A | 5' |
| ← RESTRICTION ENZYME → | | | | |
| SITE | | | | |

FIG. 13C

FIG. 16

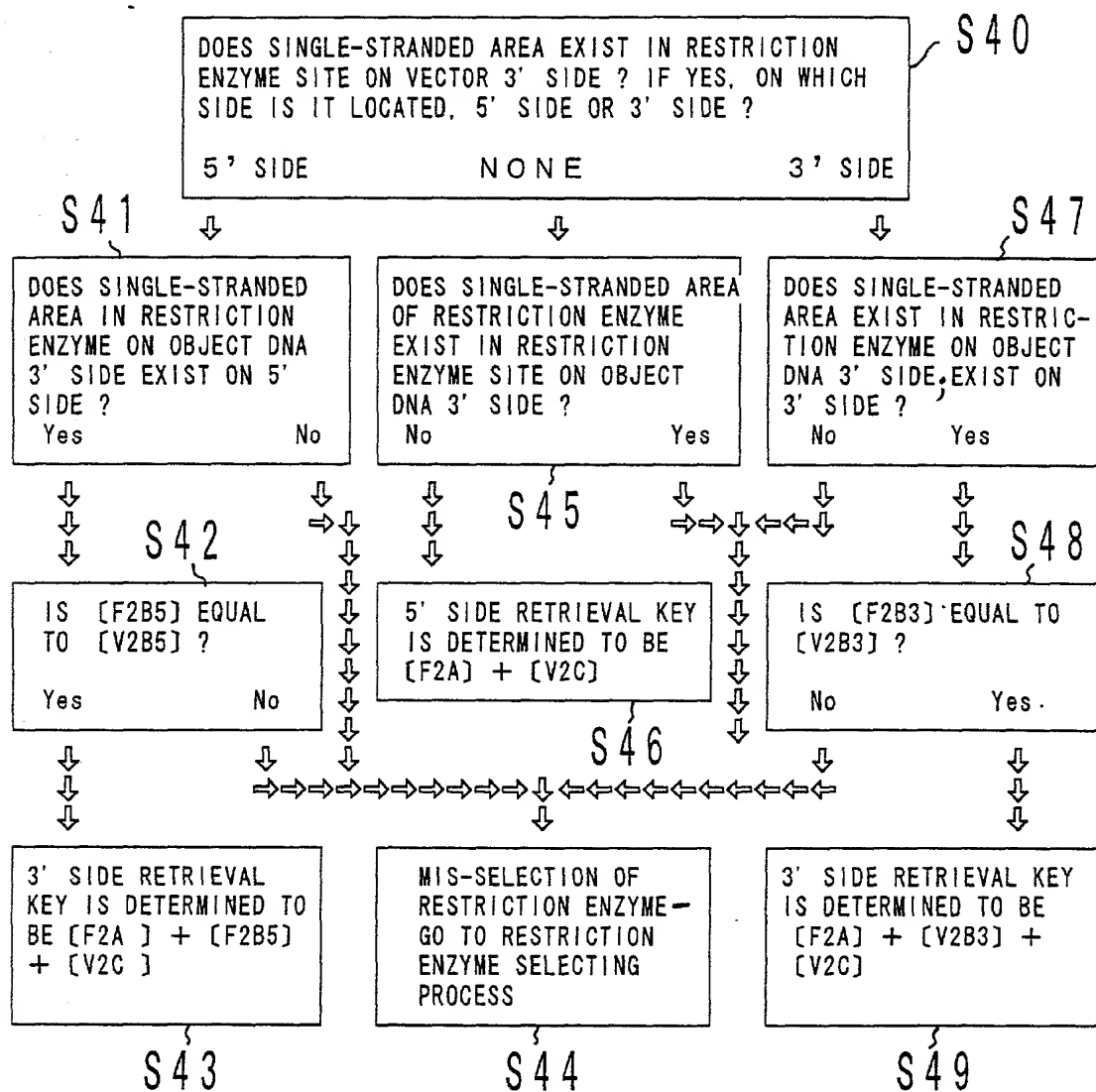


FIG. 16

[illegible]

| | |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>*****</p> <p>GTGCCAAGCTT+++++TCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAAT</p> <p>AAGCTT</p> <p>↑</p> <p>5' SIDE RETRIEVAL KEY</p> <p>(IN THIS EXAMPLE, HIND III SITE)</p> | <p>*****</p> <p>TCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAAT</p> <p>TCTAGA</p> <p>↑</p> <p>9' SIDE RETRIEVAL KEY</p> <p>(IN THIS EXAMPLE, XBA I SITE)</p> |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|

FIG. 17

SEARCHING BASE SEQUENCE OF OBJECT CLONE USING 5' SIDE RETRIEVAL KEY

S 5 1



STORING, AS LIST OF PRIMARY CANDIDATES FOR BOUNDARY PORTIONS, RETRIEVAL KEYS AND RETRIEVAL RESULTS OBTAINED AS AREAS INDICATING HOMOLOGY EXCEEDING PREDETERMINED VALUE. (LIST 5)

S 5 2

FIG. 18

SEARCHING BASE SEQUENCE OF OBJECT CLONE USING 3'
SIDE RETRIEVAL KEY

S54



STORING, AS LIST OF PRIMARY CANDIDATES FOR BOUNDARY PORTIONS, RETRIEVAL KEYS AND RETRIEVAL RESULTS OBTAINED AS AREAS INDICATING HOMOLOGY EXCEEDING PREDETERMINED VALUE. (LIST 3)

S55

F I G . 1 9

DEFINING, IN MULTIPLE CLONING SITE OF VECTOR, RESTRICTION ENZYME SITE USED IN SHEARING 5' SIDE IN MULTIPLE CLONING SITE OF VECTOR AND AREA OUTSIDE ON 5' SIDE AS 5' SIDE RESIDUAL MULTIPLE CLONING SITE (5MCS)

S 6 1



WHEN VECTOR DB CONTAINS BASE SEQUENCE OTHER THAN MULTIPLE CLONING SITE, SUM OF 5MCS AND 5 BASES ON 5' SIDE FROM 5MCS IS DEFINED AS 5' SIDE RESIDUAL VECTOR AREA (5VA). IF VECTOR DB CONTAINS ONLY BASE SEQUENCE OF MULTIPLE CLONING SITE IN VECTOR DB, THEN 5 MCS IS 5VA.

S 6 2

(A HOMOLGY CHECK IS MADE ACCORDING TO FOLLOWING FLOWCHART ON ALL ELEMENTS IN PRIMARY CANDIDATES FOR BOUNDARY PORTIONS (LIST 5) OBTAINED IN 5' SIDE HOMOLGY RETRIEVAL)

DEFINING EACH CANDIDATE IN LIST 5 AND SEQUENCE AREA OUTSIDE ON 5' SIDE AS HOMOLGY CHECK AREA (5HCA) FOR CORRESPONDING CANDIDATE

S 6 3



COMPARING NUMBER OF BASES IN 5' SIDE RESIDUAL VECTOR AREA (5VA), NUMBER OF BASES OF 5HCA, AND NUMBER OF BASES 20, AND DEFINING SMALLEST NUMBER OF BASES AS NUMBER OF BASES FOR USE IN HOMOLGY CHECK (HCB)

S 6 4



EXTRACTING HCB BASES FROM 3' SIDE OF 5VA TO CHECK HOMOLGY TO HCB BASES ON 3' SIDE OF 5HCA

S 6 5



WHEN CONSTANT HOMOLGY IS OBTAINED, EXTRACTED BASES ARE DEFINED AS SECONDARY CANDIDATES FOR 5' SIDE BOUNDARY PORTIONS.

S 6 6

F I G . 2 0

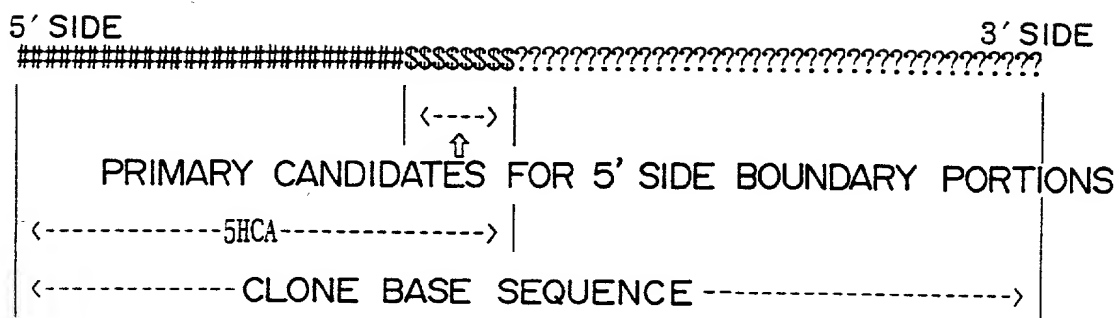


FIG. 21

DEFINING, IN MULTIPLE CLONING SITE OF VECTOR, RESTRICTION ENZYME SITE USED IN SHEARING 3' SIDE IN MULTIPLE CLONING SITE OF VECTOR AND AREA OUTSIDE ON 3' SIDE AS 3' SIDE RESIDUAL MULTIPLE CLONING SITE (3MCS)

S71



WHEN VECTOR DB CONTAINS BASE SEQUENCE OTHER THAN MULTIPLE CLONING SITE, SUM OF 3MCS AND 5 BASES ON 3' SIDE FROM 3MCS IS DEFINED AS 3' SIDE RESIDUAL VECTOR AREA (3VA). IF VECTOR DB CONTAINS ONLY BASE SEQUENCE OF MULTIPLE CLONING SITE IN VECTOR DB, THEN 3MCS IS 3VA.

S72

(A HOMOLGY CHECK IS MADE ACCORDING TO FOLLOWING FLOWCHART ON ALL ELEMENTS OF PRIMARY CANDIDATES FOR BOUNDARY PORTIONS (LIST 3) OBTAINED IN 3' SIDE HOMOLGY RETRIEVAL)

DEFINING EACH CANDIDATE IN LIST 3 AND SEQUENCE AREA OUTSIDE ON 3' SIDE AS HOMOLGY CHECK AREA (3HCA) FOR CORRESPONDING CANDIDATE

S73



COMPARING NUMBER OF BASES IN 3' SIDE RESIDUAL VECTOR AREA (3VA), NUMBER OF BASES OF 3HCA, AND NUMBER OF BASES 20, AND DEFINING SMALLEST NUMBER OF BASES AS NUMBER OF BASES FOR USE IN HOMOLGY CHECK (HCB)

S74



EXTRACTING HCB BASES FROM 5' SIDE OF 3VA TO CHECK HOMOLGY TO HCB BASES ON 5' SIDE OF 3HCA

S75



WHEN CONSTANT HOMOLGY IS OBTAINED, EXTRACTED BASES ARE DEFINED AS SECONDARY CANDIDATES FOR 3' SIDE BOUNDARY PORTIONS.

S76

FIG. 22

5' SIDE

3' SIDE

????????????????????????????????????SSSSSSSS#####

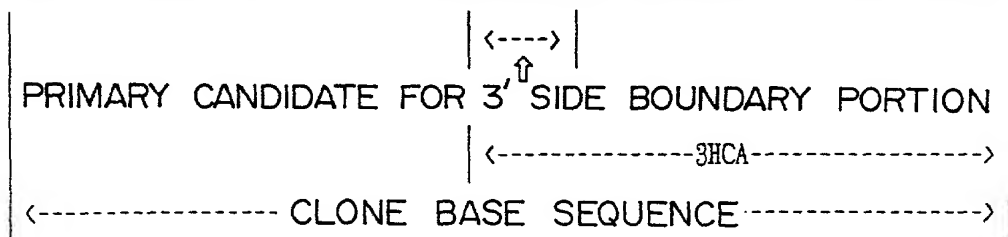


FIG. 23

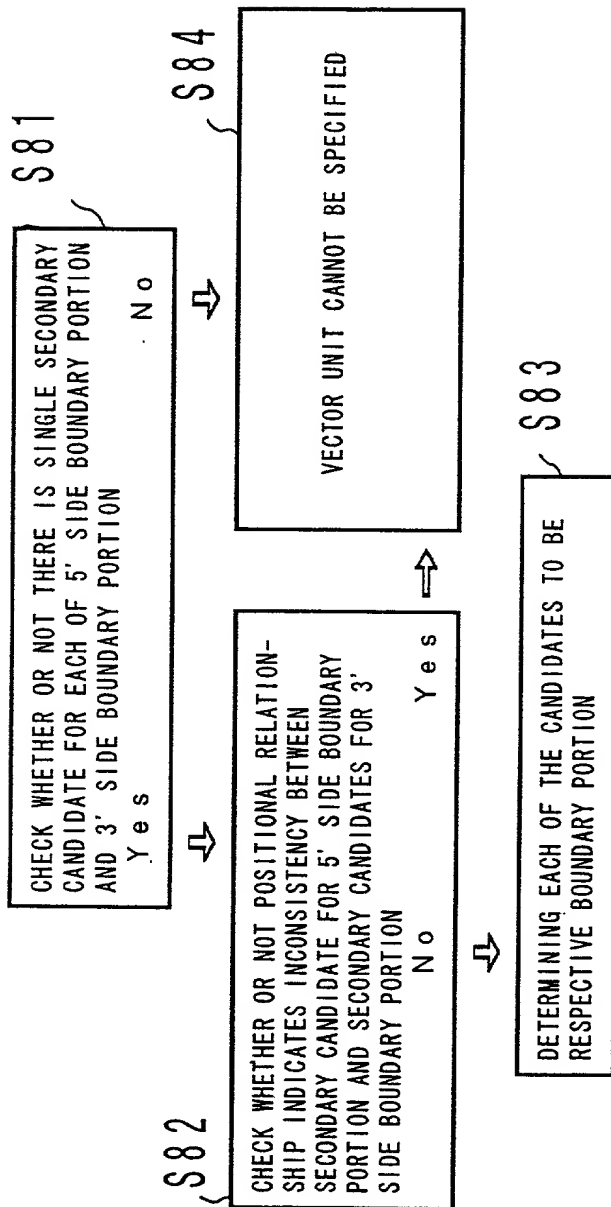


FIG. 24

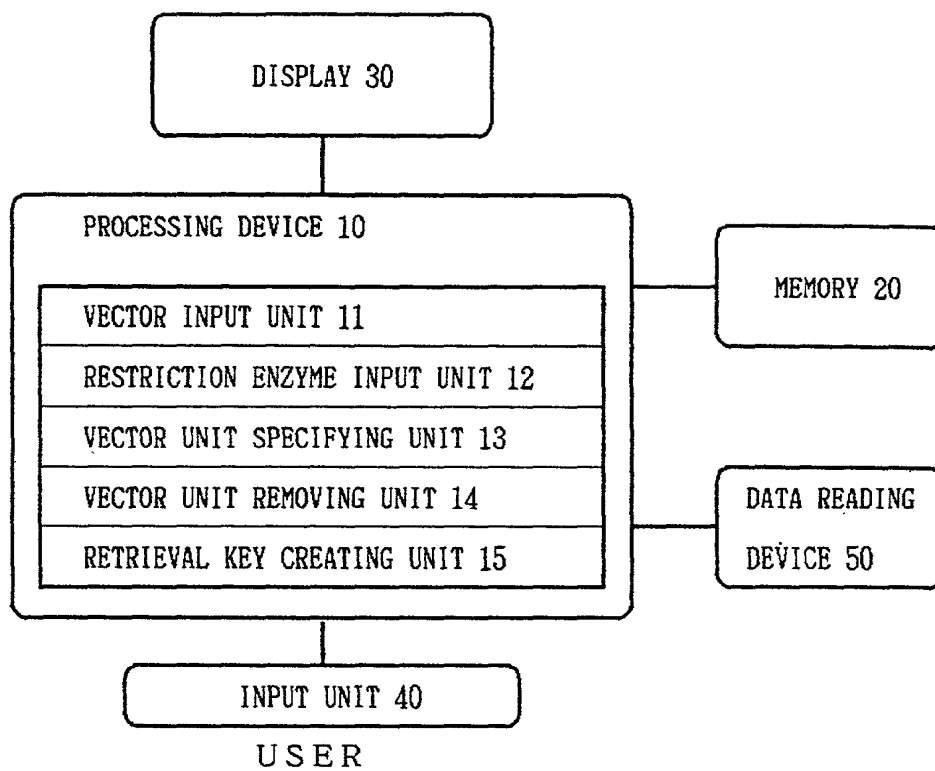


FIG. 25